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Water Research 38 (2004) 3322–3330

WATER RESEARCH

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Development of cultures capable of reducing perchlorate and nitrate in high salt solutions

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Received 7 April 2003; received in revised form 8 April 2004; accepted 23 April 2004

Abstract

An ion exchange process with biological perchlorate and nitrate destruction and reuse of spent regenerant brine has been proposed as an efficient and environmentally sound method to treat perchlorate-contaminated groundwater. A culture capable of reducing perchlorate and nitrate in spent ion exchange regenerant brine containing at least 30 g/L NaCl is needed for this to be feasible. A batch culture inoculated from activated sludge failed to acclimate to more than 15 g/L NaCl whether nitrate was present or not. A mixed culture inoculated from marine sediment was capable of simultaneously reducing 100 mg/L perchlorate and denitrifying 500 mg/L nitrate within 5 h in a synthetic medium in the presence of 30 g/L NaCl. The growth conditions to maintain this culture in a healthy state required the addition of trace metals, Na_2S, and phosphate. A second culture capable of removing 100 mg/L perchlorate from synthetic medium containing 60 g/L NaCl within 24 h was also developed.

Keywords: Perchlorate; Nitrate; Ion exchange; Brine; Anaerobic biodegradation; Denitrification; Drinking water; Groundwater

1. Introduction

Perchlorate (ClO_4^-) has become a significant contaminant of concern in surface and ground water in recent years. It is widely used in rockets, missiles and fireworks, as well as many other industrial processes [1]. Perchlorate has been known to interfere with the function of the human thyroid gland, which further affects normal metabolism and growth [2]. The EPA has recommended provisional cleanup or action levels ranging from 4 to 18 ppb. California, Nevada, Arizona, and Texas have established action levels or guides ranging from 4 to 31 ppb [2].

The perchlorate ion is nonvolatile, highly soluble, and very stable in the aqueous phase. It is not removed from water using conventional treatment including coagulation, flocculation, sedimentation, and filtration. Cost-effective treatment technologies capable of removing perchlorate to a safe level in water are urgently needed, as are methods of treating perchlorate contaminated process wastes.

Perchlorate has a high affinity for strong-base anion resins [25] and ion-exchange processes have been found to be efficient in removing perchlorate from water [3–6]. The main problem with ion-exchange processes is that they produce concentrated waste brine containing a high level of the target contaminants, in this case perchlorate and nitrate. The disposal of spent brine can be very expensive, which is the reason that Clifford and Liu [7] developed a sequencing-batch-reactor (SBR) denitrification process to treat and reuse nitrate brine containing 0.5 N NaCl. A pilot study using this ion-exchange process with batch biological denitrification and reuse of the spent brine was conducted successfully in McFarland, California in 1994 where spent brine was denitrified and reused 38 times [8]. Compared with a

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doi:10.1016/j.watres.2004.04.020

conventional ion-exchange process, brine denitrification and reuse reduced the salt consumption by 50% and waste discharge by more than 90%.

This paper deals with the development of a similar process for perchlorate treatment in ion exchange brines. Microbial perchlorate reduction under anaerobic conditions has been studied by many researchers [9–12]. Many microorganisms can reduce perchlorate to harmless chloride. Unfortunately, most known perchlorate-reducing microorganisms cannot endure high salinity in the growth media, and usually will not grow in more than 2–3% NaCl [13–15].

Coppola [16] reported that HAP-1, a strain isolated by Wallace and Attaway [17], when added in a mixed culture could reduce perchlorate in a 2–3% NaCl wastewater in the presence of high concentrations of nitrate, sulfate, ammonia, and chlorate. Rich nutrients were added to maintain the growth of the culture, which would not be recommended for an ion-exchange brine treatment process for drinking water. Logan et al. [18] screened six sources of inoculum collected from different salt-water environments for perchlorate reduction. After three months incubation, growth was observed in medium containing perchlorate and 3% NaCl with inocula from only three sources (seawater, saline lake water and biofilm/sludge). Organisms from two of these three (seawater and saline lake water) grew in 3–7% salinity in subsequent transfers. The maximum growth rate for the saline lake-water enrichment was $0.060 \pm 0.003\,d^{-1}$ at a salinity of 5% NaCl. The growth rate decreased to $0.037 \pm 0.002\,d^{-1}$ at a salinity of 11% NaCl, and no growth was observed at salinity over 13% NaCl. Only one data point showing the change of perchlorate concentration in the medium was presented in the paper, that is, perchlorate was found reduced from 592 to 45 mg/L in 3% salinity after 8 weeks while 71 mg/L cell dry weight was produced.

Typical spent brines from ion exchange processes treating water with 50–100 μg/L perchlorate and 3–20 mg/L nitrate-N would contain 2.5–10 mg/L perchlorate and 150–500 mg/L nitrate-N [3,19]. Okeke et al. [20] obtained cultures that could reduce both perchlorate and nitrate in 0–5% NaCl environments. A *Citrobacter* isolate was reported to provide the fastest nitrate and perchlorate removal in conjunction with the Perclace™ culture, removing 46.4% of the perchlorate fed to it in one week. However, because typical ion exchange columns treating perchlorate and nitrate will be exhausted and regenerated in less than 24 h [3,19] a culture must be able to remove nitrate and perchlorate within that time to avoid the need to accumulate and treat brine in large storage tanks and reactors. The objective of this research was to develop a culture capable of reducing perchlorate at high salinity (3–6% NaCl) to allow batch biological perchlorate and nitrate destruction and brine recycle for ion exchange treatment of water containing perchlorate and nitrate. This manuscript reports the development of a culture that was capable of reducing both perchlorate and nitrate in presence of 30 and 60 g/L NaCl within hours.

2. Materials and methods

In this research, two approaches were taken to the development of cultures capable of reducing perchlorate and nitrate in solutions of 30 or 60 g/L NaCl. The first was to enrich a population of perchlorate- and/or nitrate-reducing organisms from sewage, and then acclimate these to increasing salt concentrations as was done by Clifford and Liu [7]. The second was to screen six marine sediments for their use as inoculum for developing salt-tolerant perchlorate-reducing cultures. Table 1 presents a summary of the experimental and culture conditions tested in this research.

2.1. Perchlorate- and nitrate-reducing culture development from activated sludge

Activated sludge (250 mL) obtained from the City of Houston, Simms South Wastewater Treatment Plant was used to inoculate three cultures containing the basal ingredients as follows: 8 g/L NaCl, 2.5 g/L NaH_2PO_4, K_2HPO_4, and 0.001 g/L resazurin. Additionally, Medium 1 received 500 mg/L nitrate as $NaNO_3$, 100 mg/L perchlorate as $NH_4ClO_4^-$, and 1070 mg/L acetate in the form of $CH_3COONa \cdot 3H_2O$. Medium 2 received perchlorate and acetate and Medium 3 received nitrate and acetate. The reactors, operated at 22–25°C, were carefully sealed with a black-rubber stopper to exclude air, and connected to a displacement gas collector through a safety flask. Mixing was accomplished using a magnetic stir bar in the reactor. For spike feeds, cultures were fed concentrated amounts of the electron donor and acceptor in a solution of the mineral salts.

After the activated sludge cultures consistently reduced perchlorate and/or nitrate in the presence of 8 g/L NaCl, the concentration of NaCl was increased gradually from 8 to 11.5, to 15 and finally to 20 g/L. At each salt concentration, the culture was fed electron donor/acceptor several times to allow it to acclimate to the increased NaCl concentration.

2.2. Perchlorate- and nitrate-reducing culture development from marine inocula

2.2.1. Screening tests in 3% and 6% NaCl

Six anaerobic near-shore marine sediments were sampled and shipped in well sealed white-plastic buckets. They were kept refrigerated (4°C) and well sealed between uses.

Table 1
Summary of experimental conditions

ID	Media description[a]	Inoculum	Variables	NaCl (mg/L)	Mode
I Sewage 1	1	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
I Sewage 2	2	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
I Sewage 3	3	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
IIa Screening 1	4, 5, 6, 7	6 Marine sediments	Inoculum source, nitrate, yeast extract	30 g/L	Single batch
IIa Screening 2	8, 9	6 Marine sediments	Inoculum source, nitrate, yeast extract	60 g/L	Single batch
IIb Large Culture 1	6 then 4	Freeport Sediment	Perchlorate and nitrate then perchlorate alone	30 g/L	Spike and SBR
IIc Ingredients Screening	4a, 4b, 4c	Marine Culture 1	Fresh sediment, S^{-2}, trace metals, phosphate	30 g/L	Single batch
IId, e Large Culture 2	4c	Culture fed medium 4c in IIc	Perchlorate then nitrate	30 g/L	Spike and SBR
IIf Marine Culture 3	4c with 60 g/L NaCl	Freeport culture from screening exp 2	Perchlorate	60 g/L	Spike and SBR

[a] See text for media ingredients corresponding with each medium number.

Six different synthetic media were used to test the ability of the marine sediments to reduce perchlorate in the presence of 30 and 60 g/L NaCl. All six media contained the following basal ingredients per liter of deionized water; 11 g $MgCl_2 \cdot 6H_2O$, 1.4 g $CaCl_2 \cdot 2H_2O$, 0.2 g $NaHCO_3$, 0.72 g KCl to represent the major components of seawater and 0.59 g NH_4ClO_4, 10 g $NaCH_3COO \cdot 3H_2O$ to supply perchlorate and an electron donor. Sulfate was omitted from the media to prevent the growth of sulfate-reducing bacteria, which could compete with perchlorate-reducing bacteria for the electron donor. The inocula contained large amounts of reduced sulfide, which was expected to act as a sulfur source for organism growth. Four media containing the basal ingredients specified above were prepared in 30 g/L NaCl: Medium 4 contained no additions; Medium 5 contained 1 g/L yeast extract; Medium 6 contained 0.685 g/L $NaNO_3$; Medium 7 contained 1 g/L yeast extract plus 0.685 g/L $NaNO_3$. Two media containing the basal ingredients plus the following additions were made up at 60 g/L NaCl: Medium 8 contained 1 g/L yeast extract, Medium 9 contained 0.685 g/L $NaNO_3$ and 1 g/L yeast extract.

The screening tests were performed by adding 3 g of each anaerobic marine sediment to 100 mL of each medium in a 125-mL serum bottle. The dissolved oxygen in the medium was not removed, however, the headspace of the serum bottle was purged with nitrogen gas for 3 min. The serum bottles were crimp-sealed with butyl-rubber stoppers and mixed on a rotary shaker and incubated at $30 \pm 2^{\circ}C$ for at least one month. Perchlorate and nitrate (when present) were measured as described below.

2.2.2. First large culture development in 3% NaCl

A fresh sample of 3% (w/v) of Freeport #1 sediment was added to 1.5 L Medium 6 in a 2-L glass bottle reactor with a gas-collection device. The reactor was incubated at $30 \pm 2^{\circ}C$ and shaken at a rate of 150 rpm. After the initial nitrate and perchlorate in the reactor were removed, 100 mg/L perchlorate was spiked into the reactor. Nitrate was not included in subsequent spikes or feeds until a stable perchlorate reducing culture was developed. This spike-feed procedure was continued until a reproducible perchlorate reduction rate was obtained. Then the feed protocol was switched from a spiked batch reactor mode to a sequencing-batch-reactor (SBR) mode using a 30% replacement.

2.2.3. Batch medium ingredient experiments in 3% NaCl

A 10-mL inoculum from the 1.5-L perchlorate-reducing culture, which had lost activity due to SBR operation, was placed into 90 mL of medium 4a, 4b, or 4c prepared and dispensed into 125-mL serum bottles using strict anaerobic technique. Medium 4a was prepared by adding 67 mM $Na_2S \cdot 9H_2O$, to Medium 4. Medium 4b was prepared by adding 0.1 mL trace metal solution and 0.1 mL 50 g/L KH_2PO_4 to Medium 4. Medium 4c was prepared by adding 0.5 mL 67 mM $Na_2S \cdot 9H_2O$, 0.1 mL trace metal solution, and 0.1 mL

50 g/L KH_2PO_4. The trace metal solution consisted of 10 g ammonium molybdate, 0.1 g zinc sulfate, 0.3 g boric acid, 1.5 g ferrous chloride, 10 g cobalt chloride, 0.03 g magnesium chloride, 0.03 g nickel chloride, and 0.1 g aluminum potassium sulfate per liter of water.

2.2.4. Second large culture development in 3% NaCl

A second 1.5-L culture was enriched using Medium 4c by increasing the volume of the 90-mL culture from the nutrient test that had received Medium 4c by addition of fresh medium in 500 mL batches each time perchlorate was reduced to nondetect levels. The culture was maintained by spiking 100 mg/L perchlorate every three days. After every five feeds, 1 g/L sodium acetate $\cdot 3H_2O$ was spiked into the reactor as well. For several spike-feed cycles, samples were taken every two hours to measure the perchlorate concentration in the reactor.

2.2.5. Demonstration of the effect of nitrate in 3% NaCl

On the 8th feed of the second large culture, 500 mg/L nitrate was spiked with 100 mg/L perchlorate. Sodium acetate (3 g/L) was added as the electron donor for both perchlorate and nitrate reduction. Samples were again taken every 2 h, and the nitrogen gas produced in the reactor was measured in the gas collector. Both nitrate and perchlorate were spiked into the reactor for another two feeds when the perchlorate and nitrate in the current allotment of feed was reduced.

2.2.6. Perchlorate reduction at 6% NaCl

Inocula of 10 mL of the Freeport #1 culture that reduced perchlorate at 60 g/L NaCl in the screening experiments were transferred to serum bottles containing 90 mL of Medium 4c adjusted to 60 g/L NaCl. After all of the perchlorate in the medium was removed, 100 mg/L perchlorate was spiked into the culture again. This feed procedure was continued 5–6 times to allow more cell mass to grow. Then 10 mL of the culture was transferred again to 90 mL fresh medium and spiked several times. Samples were taken to test perchlorate reduction by the culture at 60 g/L NaCl.

3. Analytical methods

Samples of 1 mL (serum bottle tests) or 5 mL (1.5-L culture tests) were taken using nitrogen-flushed sterile syringes and filtered through 0.20 μm sterile syringe filters immediately after sampling, and kept in a refrigerator at 4°C if not analyzed that day.

Nitrate, sulfate, chlorate and perchlorate were measured using a Dionex DX-800 ion chromatograph configured with a GS50 gradient pump, CD25 conductivity detector, an ASRS-ULTRA suppressor, and an AS40 automated sampler. The suppressor was set at 300 mA. Separation was obtained using a Dionex IonPac AS16 anion analytical column (4 mm × 250 mm) mm) and an AS16 guard column (4 mm × 50 mm). A 25-μL sample loop was used to measure perchlorate concentration higher than 1 mg/L. The sample loop was switched to 1000 μL to measure lower perchlorate concentrations. The detection limit for perchlorate was 5 ppb in de-ionized water and 500 ppb in the presence of ⩾8 g/L NaCl concentration. A gradient eluent was delivered in order to separate all peaks: Initially, a flow of 5 mM KOH was maintained for 2 min at a flow rate of 1.0 mL/min. The eluent KOH composition was changed to 10 mM in a linear gradient from 2 min to 14 min with the flow rate unchanged. A linear gradient was then used to change the eluent composition to 55 mM KOH from 14 min to 20 min while the flow rate was increased to 1.5 mL/min at 20 min. These conditions were held constant from 20 to 27 min. All water used was de-ionized, reagent grade with 18 MΩ cm resistivity.

Nitrite was analyzed by absorbance using the method described in *Methods of Seawater Analysis* [26] because it could not be resolved from the chloride peak during IC analysis. The absorbance was measured in 1-cm cuvettes at 540 nm with Lambda 3B UV/VIS spectrophotometer, Perkin-Elmer Corporation.

4. Results

4.1. Culture development from activated sludge

The three cultures developed from activated sludge were fed acetate as the electron donor and (1) nitrate and perchlorate, or (2) perchlorate only, or (3) nitrate only as the added electron acceptors at an initial NaCl concentration of 8 g/L.

All three cultures were able to adapt quickly to the removal of perchlorate and nitrate from the media when the NaCl concentration was 8 g/L. The two cultures fed perchlorate only could not tolerate more than 15 g/L NaCl in the media. The culture fed with perchlorate and nitrate never showed recovery from any step increase in salt concentration. These results suggest that the presence of nitrate may have some negative effect on perchlorate reduction at higher salt concentrations. Because the culture fed with perchlorate alone did not acclimate to more than 15 g/L NaCl, there must be other physiological problems as well.

The fact that neither culture was able to acclimate to the targeted 30 g/L NaCl concentration whether or not nitrate was present, demonstrates that the sewage-sludge-acclimation approach was not a successful strategy to obtain a culture capable of reducing perchorate and nitrate at 30 g/L NaCl. The control culture that was fed only nitrate was able to adapt to 30 g/L NaCl with no apparent problems.

4.2. Culture development from marine sediment

4.2.1. Screening experiments in 3% and 6% NaCl

A 30-day sample of six marine sediments incubated in synthetic media with 30 or 60 g/L NaCl revealed that the organisms in only three of the sediments—Freeport #1, Fourchon #1 sand Fourchon #3—were capable of reducing perchlorate (Fig. 1). All six sediments reduced at least 98% of the nitrate in all of the media having nitrate (results not shown).

In the presence of 60 g/L NaCl (Fig. 2), no perchlorate reduction was observed by the 30-day sampling period while at least 98% of the nitrate in all of the media that contained nitrate was reduced. By the 45-day sample, the Freeport #1, Fourchon #1 and Fourchon #3 sediments showed perchlorate reduction. Again, the other three sediments did not show much perchlorate reduction.

4.2.2. First large culture development in 3% NaCl

The Freeport #1 sediment was selected as the most consistent inoculum and Medium 4 containing perchlorate and nitrate at 30 g/L NaCl was selected as the growth medium to enrich a larger-scale perchlorate reducing culture. This 1.5-L Freeport #1 culture experienced a 28-day lag period, but was then able to reduce 510 mg/L perchlorate to 4.93 mg/L within 56 days. Nitrate was reduced within the first week of incubation. Thereafter, along with each spike feed of 100 mg/L perchlorate, the perchlorate reduction rate

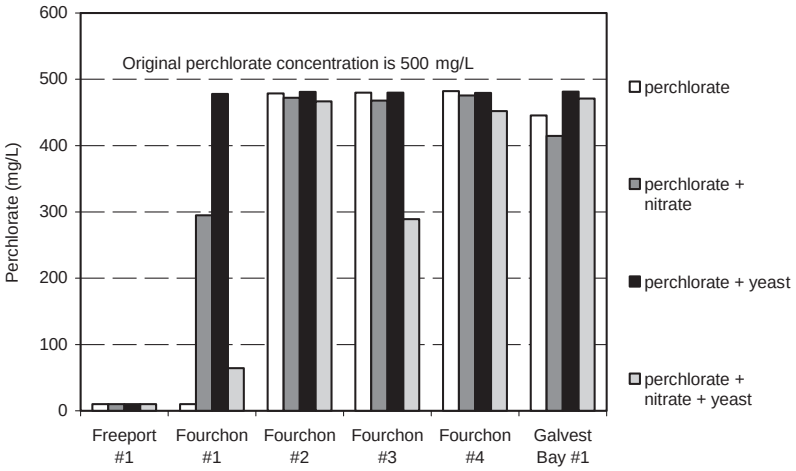

Fig. 1. Perchlorate concentration remaining after 30 days in perchlorate media containing 30 g/L NaCl inoculated with six different marine sediments $T = 30^{\circ}C$.

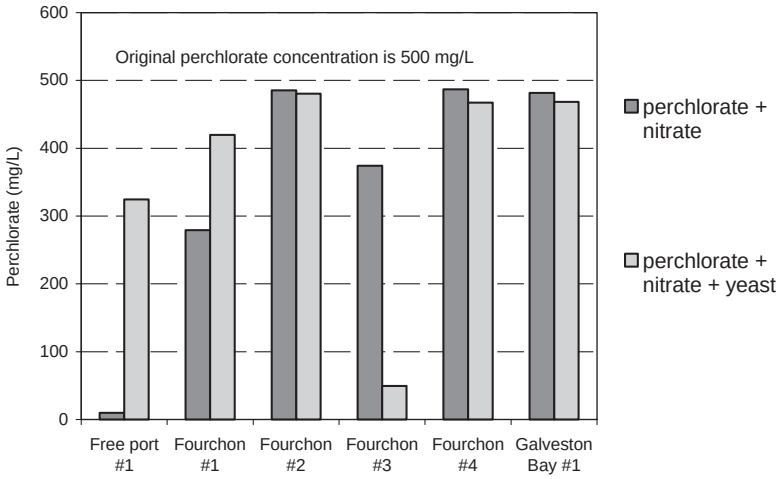

Fig. 2. Perchlorate concentration remaining after 45 days in perchlorate media containing 60 g/L NaCl inoculated with six different marine sediments $T = 30^{\circ}C$.

increased, and an increase of the biomass was observed in the reactor. After 3–4 perchlorate spikes, the culture could remove 90% of perchlorate fed in the medium within 30 h.

In order to simulate the ion-exchange brine reuse process the culture was then operated under SBR mode. This dramatically decreased perchlorate reduction. It took more than six days to reduce the same amount of perchlorate for the first feed in the SBR mode, and more than nine days for the second feed using SBR conditions. This suggested that some ingredient in the initial mud inoculum that was important for perchlorate reduction by the culture was depleted during medium replacement.

4.2.3. Batch medium ingredient experiments in 3% NaCl

The original Freeport 1 marine sediment was rich, black, and very anaerobic. To determine if there were abiotic factors present in the mud that enabled the culture to reduce perchlorate rapidly, fresh, autoclaved Freeport marine sediment was added to duplicate transfers of the ineffective large culture to determine if this could return the culture to a rapid perchlorate reduction rate. Adding the autoclaved sediment had a beneficial effect. The culture containing sediment-amended medium had less perchlorate remaining after a five-day incubation period than the controls. This trend was again observed after a second spike of perchlorate into the cultures (not shown).

The most obvious abiotic factors in the sediment that could be beneficial to the culture were sulfide or other mineral nutrients. To determine which components might be responsible for the beneficial effect, Na_2S and trace minerals were added to the culture. The addition of phosphate as a traditional biological nutrient was also examined. The addition of Na_2S, trace metals and phosphate together caused the most beneficial effect. The addition of Na_2S alone somewhat improved perchlorate reduction, whereas trace metals and phosphate only had no beneficial effect (results not shown). Na_2S provides sulfur for microbial growth, scavenges oxygen, and reduces the redox potential in the culture. Lower redox potential is helpful to anaerobic perchlorate reduction. Trace metals and phosphate are important to the bacteria's growth and metabolism, especially for bacteria growing in strict environments (anaerobic and high saline). From these results, this marine culture needs both low redox potential and trace metals to reduce perchlorate.

4.2.4. Second large culture development in 3% NaCl

The culture growing in trace metal-, phosphate- and Na_2S- amended Medium 4c was used to create another 1.5 L culture. After several spike feeds of ~100 mg/L perchlorate, this culture was capable of removing 70–100 mg/L perchlorate within 8 h (Fig. 3). After 48 daily

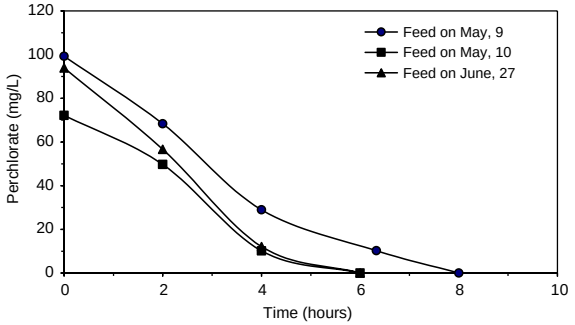

Fig. 3. Perchlorate reduction by the second large culture fed Medium 4c, $T = 30°C$.

SBR feedings of Medium 4c from this point, samples were collected every two hours and analyzed for perchlorate during one react phase. The results showed that the culture performance was stable (Fig. 3). This culture continued to reduce its allotment of perchlorate in each daily feed or SBR operation for two months.

4.2.5. Demonstration of the effect of nitrate in 3% NaCl

The ability of the culture that could degrade perchlorate successfully in 30 g/L NaCl to reduce perchlorate in the presence of nitrate was also tested. Fig. 4 presents the perchlorate and nitrate concentrations for the last feed with perchlorate alone and the first feed with perchlorate (97 mg/L) and nitrate (558 mg/L) together. The addition of 9× as much nitrate (molar basis) as perchlorate did not effect the perchlorate reduction by the culture. Perchlorate was reduced within 6 h with or without the presence of nitrate. The perchlorate reduction curves were modeled with first order kinetics and the k-values were 0.627/h and 0.514/h. Nitrate (9 mM) was also reduced within 10 h.

The culture adapted to denitrification very quickly by the third spike feed, when the 92 mg/L perchlorate and 539 mg/L nitrate were both reduced (98%) within 5 h. At least 153 mL nitrogen gas was collected, which is comparable to the theoretical gas production (147–155 mL assuming 1 mol NO_3^- was converted to 0.45–0.48 mol nitrogen gas, 30°C, one atm. total pressure, and water-saturated air). This suggests that this marine culture can denitrify at a rapid rate along with the reduction of perchlorate.

A microscopic examination of a Gram-stained sample of the culture revealed that this was not a pure culture but the majority of the organisms present in the culture were Gram-negative, slightly curved rods.

4.2.6. Perchlorate reduction at 60 g/L NaCl

Although 30 g/L NaCl can be used to regenerate the perchlorate-spent resin, the preferred concentration of

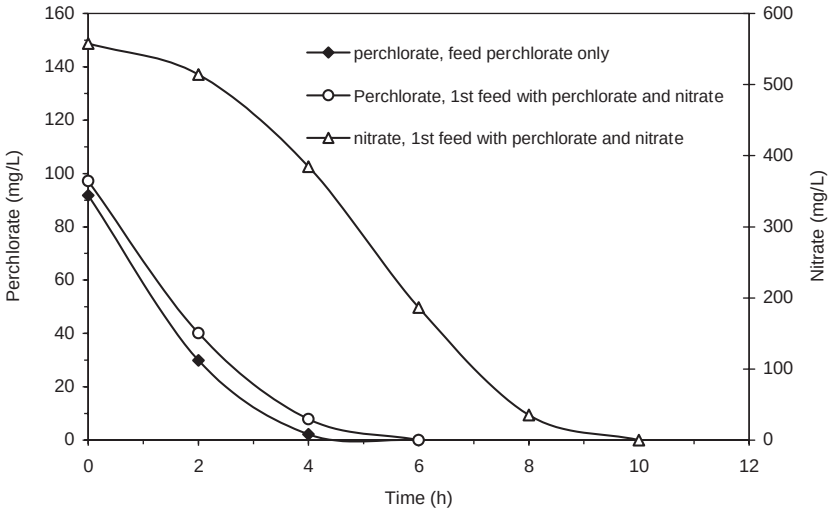

Fig. 4. Culture performance in 3% NaCl when fed perchlorate alone and fed perchlorate and nitrate together for the first time in Medium 4c, $T = 30^{\circ}C$.

NaCl in the ion-exchange brine is 60 g/L (6%) or higher. Initial batch screening tests provided a culture that was initially capable of reducing perchlorate in a medium that contained 60 g/L NaCl within 45 days, but lost the capability in the subsequent transfer to fresh medium with 60 g/L NaCl. Once it was learned that the 30 g/L culture required sulfide, trace metals and phosphate, these ingredients were added to revive the culture in the 60 g/L medium. After 1 or 2 transfers to fresh Medium 4c adjusted to 60 g/L NaCl and several spike feeds of 100 mg/L perchlorate, a stable culture capable of reducing perchlorate within 1 day at 60 g/L NaCl was obtained. The culture was capable of removing more than 90% of 80–100 mg/L perchlorate within at most 29 h (Fig. 5). The data from the curves presented in Fig. 5 were modeled using zero-order kinetics. The average perchlorate degradation rate was 3.61 mg/L h. The fit to a zero-order curve suggests that there are low numbers of perchlorate-degrading microbes present in this culture so the degradation rate is saturated even at low perchlorate concentrations.

The pathway of perchlorate degradation involves the sequential reduction of perchlorate to chlorate, chlorite, and finally, chloride [12,13,21–24]. The analytical method used allowed the detection and quantification of perchlorate, and chlorate, but not chlorite. The chloride produced from the reduction of perchlorate could not be quantified because of high background of NaCl (3–6%) in the media. For the culture enriched from the Freeport #1 sediment, chlorate was observed only transiently in early enrichment cultures, but was never observed in mature cultures. The completion of the respiration of perchlorate can be inferred by a change in redox potential indicated by the color change

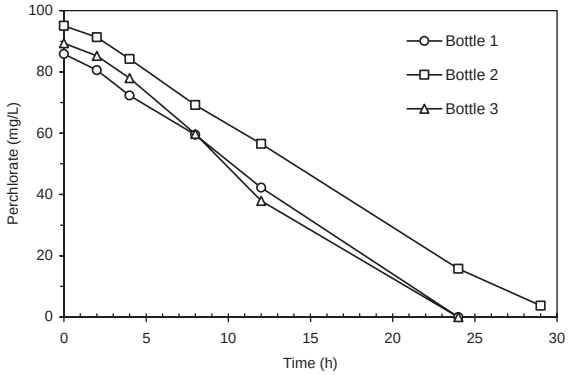

Fig. 5. Perchlorate reduction in the presence of 60 g/L NaCl. The culture was fed Medium 4c adjusted to 60 g/L NaCl. Bottle 1 is the first transferred culture spiked with additional perchlorate six times; Bottles 2 and 3 are both the second transferred cultures spiked twice with additional perchlorate. The cultures were incubated at 30°C.

of resazurin due to O_2 produced in the final reaction. This was observed, again, in enrichment cultures, but rarely in the mature cultures. This does not mean that complete metabolism was not achieved but only that the O_2 was removed as fast as it was produced.

An electron balance was conducted for electron use by the perchlorate-reducing culture enriched from Freeport #1 sediment. In five spike feed cycles, 40 meq of perchlorate (ClO_4^- to Cl^-) were fed and 59 meq of acetate (CH_3COO^- to CO_2) were used. The electron equivalence of acetate is higher than the equivalence of perchlorate, which supports a total reduction of perchlorate to chloride and indicates that acetate was also used for biomass generation.

5. Conclusion

Cultures inoculated with activated sludge were capable of reducing perchlorate at low salt concentration (<15 g/L) but not able to acclimate to as little as 20 g/L NaCl in the feed solution whether or not nitrate was present. The perchlorate reduction was significantly effected by the increase of salt concentration. Nitrate reduction was not as sensitive to salt concentration.

Two cultures capable of degrading perchlorate and nitrate in high salt solutions were developed from marine inoculum. One culture is capable of reducing up to 100 mg/L perchlorate and 500 mg/L nitrate within 5 h in the presence of 30 g/L NaCl. The other is capable of reducing 100 mg/L perchlorate in the presence of 60 g/L NaCl within 24 h. The growth conditions to maintain these cultures in a healthy state require the maintenance of strictly anaerobic conditions and the addition of trace metals, Na_2S and phosphate.

Other researchers have described the destruction of perchlorate in high salt solutions or in brine. This is the first research to describe the rapid simultaneous destruction of both perchlorate and nitrate in high concentration salt solutions of the type that will occur in ion-exchange processes for perchlorate removal. Although further research is needed to better understand the microbiological makeup of the culture, it is promising that the enriched marine cultures can be used to remove perchlorate and nitrate from 30 or 60 g/L spent ion-exchange regenerant brine within a 24-h period.

Acknowledgements

The funding for this research was provided by a partnership between the EPA, the EAST Valley Water District, and AwwaRF.

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